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Diazotrophic plant growth-promotion bacteria and sugarcane straw levels: Effects on sugarcane productivity and on soil enzymatic and microbiological activities

R.F. Vieira*, A.J.B. Luiz and N.P. Ramos

Embrapa Meio Ambiente, Soil Biogeochemical Departament, CP 69, CEP 13918-110, Jaguariúna, São Paulo, Brazil
*Corresponding author: rosana.vieira@embrapa.br

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Abstract. Due to the rising demand for bioenergy, a certain amount of sugarcane straw is often removed for ethanol production, but this practice may compromise health soil. In addition to the amount of straw, growing concern with the environment has made the use of diazotrophic plant-growth promotion bacteria (DPGPB) an alternative to the use of nitrogen fertilizers. A research was designed with the aim of evaluating the effect of different amounts of straw kept on the soil on soil microbial and enzymatic parameters and on sugarcane yield during the first ratoon. The field experiment was carried out in randomized blocks, using nitrogen fertilizer (NF) or DPGPB (BI), both with four straw levels. The parameters evaluated were: β-glucosidase (GA), urease (UR) and acid phosphatase (APA) activities, fluorescein diacetate activity, basal soil respiration, microbial biomass N, total C, total N and labile carbon. Responses were not obtained for various parameters with respect to the straw levels which was attributed to the short experimental period. In general, the evaluated parameters were always higher in the BI treatment, except for APA, which was lower in this treatment. This fact could be attributed to the greater capacity of the inoculated plants to make phosphorus available to the plants, which could reduce APA. The absence of a response for sugarcane yield between the NF and BI treatments demonstrates the beneficial effect of the bacteria in the inoculant in suppressing plants with adequate nitrogen contents. Greater yields were obtained for the L64 and L100 straw levels with no significant difference. This demonstrates the adequateness of removing part of the straw to produce second generation ethanol.

Key words: plant growth-promoting microorganisms, nitrogen fertilizer, soil microbial activity, soil enzymes, bioenergy.

INTRODUCTION

Sugarcane (*Saccharum* spp.) is the most important crop for Brazilian production of renewable energy and is also a feedstock for 80% of sugar production worldwide (Yang et al., 2021). Brazil is the world's largest sugarcane producer, with 598 million t of stalk and 26 billion L of ethanol produced in 8.3 million hectares during the 2021/2022 season (CONAB, 2023). Starting in 1997, but intensified in the last decade due to environmental, agronomic and economic reasons, the sugarcane harvesting system

changed from manual slashing after burning of unwanted leaves to mechanical harvest without burning leaving the dry leaves and green tops (straw, also known as trash) on the ground, in a system called green cane management. This management promotes several significant benefits for the crop and soil (Bordonal et al., 2018a). In Brazil, almost 89.4% of the areas cultivated with sugarcane had been mechanized by 2021/2022, with no burning (CONAB. 2023).

In Brazil, between 8 and 30 Mg ha^{-1} $year^{-1}$ of straw on a dry weight basis, are left in the field after harvesting (Franco et al., 2013; Carvalho et al., 2013). The high energetic potential of sugarcane straw has generated interest on its removal for the production of energy or ethanol by enzymatic hydrolysis i.e. second generation ethanol (Carvalho et al., 2017; Lisboa et al., 2017). Sugarcane straw is also being used for bioelectricity cogeneration (Bordonal et al., 2018b). However, the indiscriminate removal of the straw can jeopardize soil quality and consequently reduce crop yield (Lisboa et al., 2019) causing considerable controversy with respect to the establishment of recommended levels for its removal from the field.

Nitrogen is the second most required element for sugarcane, only behind potassium (K) (Cherubin et al., 2018; Otto et al., 2019). The input of *N*-fertilizers used in the Brazilian sugarcane production systems is still low compared to other regions of the world, which have similar yields (Robinson et al., 2011; Baptista et al., 2014). However, the extensive cultivation area places this culture in second place in Brazil with respect to demand for nitrogenated fertilizers, responsible for 22% of the country's *N*-fertilizers consumption (IFA, 2013). In addition, the N-use efficiency in sugarcane production has been reported to be in the range of 6–40% (Oliveira et al., 2002), i.e. more than 60% of applied *N*-fertilizer can be lost to the environment. According to Otto et al. (2016) the area used for sugarcane cultivation is expected to continue to expand as the domestic demand and consumption of ethanol products increase over coming decades. According to those authors since this expansion will occur primarily in areas with low fertility soils, substantial N inputs will be required to generate high yields. Some projections indicate that both the sugarcane area and production will reach 11.5 Mha and 884 million Mg, respectively, in 2024 (OECD/FAO, 2015). This is particularly worrying if one considers that *N*-fertilizers have a high potential for contaminating air and water, challenging the sustainable production of biofuels (Erisman et al., 2010).

An important strategy to remediate the problem associated with synthetic *N*-fertilizers is the use of diazotrophic plant growth-promotion bacteria (DPGPB). Such microorganisms represent an important agricultural and ecological strategy to guarantee economic gains with less damage to the environment (Herrera et al., 2016). Sugarcane can associate with a great diversity of DPGPB, which could benefit the nitrogen nutrition of the plant both directly and indirectly (Pereira et al., 2019). Field inoculation studies with DPGPB showed that the yield response varied with sugarcane varieties, and was due to increases in the input of N_2 fixation and, possibly, to other growth-promoting effects (Schultz et al., 2012), such as the production of phytohormones (Bashan et al., 2004), phosphate solubilization or the retention of essential nutrients in the soil (Yadav et al., 2009).

The potential contribution of DPGPB in the biological fixation of N_2 was reported to be up to 70% of total nitrogen incorporated by some sugarcane varieties (Urquiaga et al., 1992). Other studies that aimed at quantifying the contribution of biological N_2 fixation by this crop, reported that approximately 50% of the total N accumulated in

plant tissues comes from the air (Urquiaga et al., 2012). Schultz et al. (2012) reported that the inoculation of sugarcane with diazotrophic bacteria caused increases in the development with productivity similar to the addition of 120 kg N ha^{-1} for the sugarcane variety RB867515. Plants inoculated with five different bacterial species (*Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae, Herbaspirillum rubrisubalbicans, Azospirillum amazonense*, and *Burkholderia* sp. and those inoculated with a mixture of the two *Herbaspirillum* spp species, increased the dry matter yields by 35% and 26%, respectively, as compared to the non-inoculated control (Oliveira et al., 2002). Pereira et al. (2019) obtained results which evidenced the viability of using diazotrophic plant-growth promoting bacteria to increase the productivity of sugarcane and possibly reduce nitrogen fertilization. Although the inoculation with DPGPB is being consolidated as a technology with great potential to promote sugarcane growth, the effects of these microorganisms in areas with different amounts of straw left on the soil are still scarcely available. This knowledge is important since the removal of part of the straw can vary the soil temperature and water content in addition to affecting several physical, chemical and biological processes in the plant rhizophere (Ruiz Corrêa et al., 2019), which could influence the potential of DPGPB to promote sugarcane growth and yield. Similarly, the effects of removing part of the straw associated with inoculation of DPGPB, on the microbiological and enzymatic processes in the soil were also not found, despite the importance of microorganisms in the dynamics of the nutrients (Zhao et al., 2014). The use of DPGPB can promotes changes in the richness and composition of the microbial community (Pellegrini et al., 2021) and these factors can influence the microbiological processes in the soil.

Soil enzymes have been suggested as one of the potential biological indicators of soil quality because of their relationship with soil biology, ease of measurement, and rapid response to changes in soil management (Flieβbach et al., 2007). Soil enzyme activity is one of the first soil properties that is altered when the system is disturbed, and controls both the supply of nutrients to plants and microbial growth. Amongst these enzymes one could cite β-glucosidase, urease and acid phosphatase, involved in the carbon (C), nitrogen (N) and phosphrus (P) cycles, respectively. β-glucosidase carries out the final step in the degradation of cellulose, liberating glucose, which is the main carbon source for the soil microorganisms (Wang et al., 2010). Urease plays an important role in the nitrogen cycle, since it hydrolyses urea-type compounds to CO_2 and ammoniacal N (Zhang et al., 2014). Phosphatases are a group of enzymes that can hydrolyze organic phosphate compounds, being essential in the mineralization and cycling of phosphorus in the environment (Jing et al., 2020). The enzymatic method determining the hydrolytic activity of fluorescein diacetate has been widely used as an indicator of soil quality and measures the general activity of microorganisms as well as the basal respiration (Adam & Duncan 2001; Schumacher et al., 2015). The rate of FDA hydrolysis has been considered as an index of microbial activity because its hydrolysis is carried out by active cells using lipases, proteases and esterases. The advantage of this method is its simplicity, sensitivity and rapidity (Dzionek et al., 2018).

This research was carried out with the objective of answering the following questions: can the inoculation of sugarcane with DPGPB promote changes in the microbiological and enzymatic activities of the soil? How can the amounts of straw left on the soil after sugarcane harvesting affect these possible changes? The present study, to the best of our knowledge, is the first report investigating all these factors in a concomitant.

MATERIALS AND METHODS

Experimental site and treatments

The research was carried out between October 2012 and April 2015, corresponding to the period of reform of the sugarcane and the 1st ratoon. The sugarcane plantation is located in the municipality of Guaíra, São Paulo State, Brazil (lat. 20º 19' 06" S; long. 48º 18' 38" W), in an area with a 24-year history of cultivating sugarcane, being the last eight years without burning and using mechanized harvesting (Fig. 1). The predominant climatic condition in the region is tropical (Aw) according to Köppen´s classification, characterized by hot summers and a dry season from May to September. The mean annual air temperature varies from 16 °C to 33 °C, while the mean monthly precipitation varies from 13 mm to 299 mm (Fig 2). The soil type is Acriferic Red Latosol with 21% of sand, 15% of silt and 64% of clay. Table 1 presents some soil chemical characteristics of the soil (Embrapa, 2011).

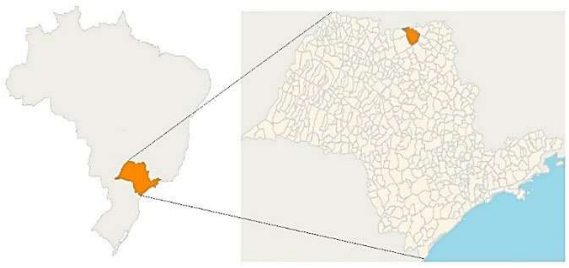

Figure 1. Location of Guaira city in the state of São Paulo, Brazil.

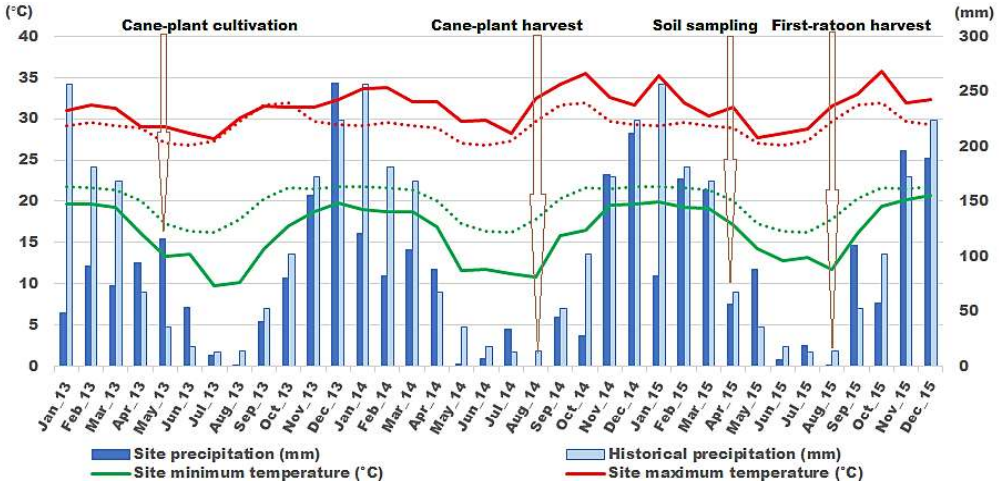

Figure 2. Climatic conditions during the experiment.

During the reform period, soybean was cultivated using a no-till system. The legume was seeded in November, 2012, after inoculation of the seeds with different species of *Bradyrhizobium* spp. Before seeding, the soil was fertilized with triple superphosphate (80 kg ha^{-1} of P_2O_5) and potassium chloride (90 kg ha^{-1} of K_2O). The soybeans were harvested in March 2013. The sugarcane was planted in May 2013 using the variety IACSP95-5000. About 20 sugarcane buds were used per linear meter, obtained from mechanically harvested chopped stalks and deposited at the bottom of the planting furrow at a depth of 35 cm. During the cane-plant cultivation period, both

sugarcane straw from the previous cycle and straw from the recently harvested soybean (4.7 Mg ha^{-1} on a dry weight basis) were left on the soil.

Table 1. Soil chemical analysis

Treatments	TC	pH (H_2O)	P	S	K	Ca	Mg	SB	H+Al	CTC	V%
NF	16.3a	5.2a	23.0b	28.5ab	3.7b	40.3a	12.5a	56.4b	33.5a	89.9a	62.4ab
BI	17.8a	5.4a	27.5ab	26.0a	4.7a	41.5a	12.5a	58.7ab	28.5b	87.2ab	67.0a

TC, total carbon, g kg^{-1}; P, mg dm^{-3}; K, Ca, Mg, $mmol_c$ dm^{-3}; SB, sum of bases, $cmol_c$ dm^{-3}; H + Al, $cmol_c$ dm^{-3}; CEC, cation exchange capacity, $cmol_c$ dm^{-3}; V, saturation of bases, %. The soil pH was determined at a soil-to-water ratio of 1:2.5. The total C (TC) contents were determined using an elementary C and N analyzer (Truspec-Leco). BI, sugarcane inoculated with DPGPB; NF, sugarcane with nitrogen fertilizer applications. Values within the same columm followed by different letters indicate significant differences at $P \leq 0.05$ ($n = 4$).

For the cultivation of the cane-plant all plots received 32 kg N ha^{-1} and 156 kg P_2O_5 ha^{-1} in the form of mono-ammonium phosphate, placed at the bottom of the planting furrow. After the plants reaching a height of 40 cm, 117 kg ha^{-1} de K_2O in the form of potassium chloride was applied as side dressing. The site was then divided into two large parallel areas where the treatments were implemented, that is, one with nitrogen fertilizer (NF) and the other where the inoculant (BI) would be applied. The NF area received an additional of 100 kg N ha^{-1} in the form of ammonium nitrate as side dressing. The other area received an inoculant containing diazotrophic plant growth-promoting bacteria (DPGPB) sprinkled on the stalks. Forty days after the first inoculation, a new application of the inoculant was made on the leaves. The soil acidity correction was made in the last ratoon before the reform period.

For the inoculations a total of 1,250 g of peat inoculant (10^8 cells g^{-1} peat) was diluted in 32 L of water, and 4 L of this were transferred to the tank of a coastal sprayer with a capacity for 12 L and the volume completed with 5 L of water to give a volume sufficient for the useable area of a plot. An inoculant composed of five strains isolated from different sugarcane varieties in Brazil and deposited in Embrapa Agrobiology Culture Collection (Seropédica, Rio de Janeiro, Brazil) was used, that is, *Gluconacetobacter diazotrophicus* (strain PAL5^T-BR 11281^T), *Herbaspirillum seropedicae* (HRC54-BR 11335), *Herbaspirillum rubrisubalbicans* (HCC103-BR11504), *Nitrospirillum amazonense* (Cbamc-BR11145) (formerly *Azospirillum*) and *Paraburkholderia tropica* (PPe8^T-BR11366^T) (formerly *Burkholderia)* (Oliveira et al. 2006).

After the cane-plant harvest in August 2014, the treatments with the different straw levels were established as a randomized complete block design, in a factorial 2×4, with four replications and the following treatments: inoculation with diazotrophic plant growth-promotion bacteria (BI) or the use of nitrogen fertilizer (NF), both with four different amounts of straw left on the soil, giving a total of 32 plots. The four sugarcane straw levels were established by regulating the exit of material from the primary and secondary ventilators of the harvester, as follow. 0% (L0, 0 kg ha^{-1}), 36% (L36, 4,300 kg ha^{-1}), 64% (L64, 7,400 kg ha^{-1}) and 100% (L100, 11,500 kg ha^{-1}). The percentages refer to the amounts of straw left on the soil in relation to the total amount. Each plot (10 m × 15 m) was comprised of 10 sugarcane rows spaced at 1.5 m. The NF treatment plots received as side dressing, 120 kg N ha^{-1} in the form of ammonium nitrate and 150 kg ha^{-1} of K_2O in the form of potassium chloride, 75 days

after harvesting the cane-plant. For the BI treatment plots the inoculant was sprayed on the plants associated with the addition of 150 kg ha^{-1} de K_2O. Together with this fertilization the soil acidity was corrected again using 2.0 Mg ha^{-1} of dolomite limestone and 1.3 Mg ha^{-1} of silicate. The parameters described below were evaluated eight months after the plant-cane harvest (April, 2015).

Soil exoenzyme assays

To carry out the enzymatic analyses, six sub-samples of soil were collected from each plot at a depth of 0–15 cm, and 20 cm away from the plant and mixed to form a bulk sample. The samples were stored at 4 °C in the laboratory after being passed through a 4-mm mesh sieve and analyzed within 24 hrs. The acid phosphatase (APA) and the β-glucosidase (GA) activities were assayed according to Tabatabai (1994), measuring the release of *p*-nitrophenol after incubating the soil with *p*-nitrophenyl phosphate and *p*-nitrophenyl-β-D-glucoside, as substrate, respectively. The calibration curves were generated using 0.1 M Tris buffer (pH = 12) with *ρ*-nitrophenol. The urease activity (UA) was measured according to Nannipieri et al. (1978), measuring the ammonium released after incubating the soil with urea, for 2h at 37 °C. The calibration curve was generated using NH_4Cl. The NH_4^+ quantified during the UA assays was determined using a Flow Injection Analyzer (FIAS 300-Perkin Elmer).

Microbial activity and microbial biomass N assays

The microbial biomass N (N_{mic}) was quantified according to Brookes et al. (1985) based on the difference between the N extracted with 0.5 M K_2SO_4 from chloroform fumigated and unfumigated soil samples, using a *Kc* value of 0.54. The quantity of C extracted from unfumigated soil was used as a measure of the labile C fraction of the soil (K_2SO_4-C) (El-Naggar et al. 2015) and analyzed using a Shimadzu 5000A soluble C analyzer.

The total microbial activities were evaluated by the determination of soil basal respiration (BR) and using the fluorescein diacetate (FDA) analysis. For the BR determination the soil was incubated for 15 days, at 25 °C ± 2 °C. The CO_2 released from the samples was captured by a NaOH solution (0.5 mol L^{-1}), which was titrated with HCl (0.5 mol L^{-1}) using phenolphthalein (1%) as indicator. The FDA hydrolysis was determined as described by Adam & Duncan (2001). The moisture content was determined in order to report the analytical results on a dry soil basis.

Sugarcane yield

Cane-plant and the first ratoon were harvested in August, 2014 and in August, 2015, respectively. Stalks yields were measured in an area of 3 m^2 within each plot. The stalks were removed, weighed (kg) and the results were extrapolated to t ha^{-1}.

Statistical analysis

The variables were submitted to an analysis of variance to determine the effect of the treatments, and *Tukey's test* ($P \leq 0.05$) was applied to compare the means when the effect was significant. When no straw effect was found the data were only presented for BI and NF treatments. Pearson correlation analysis was also carried out between the different variables. The PROC GLM routine from the SAS program was used (SAS 2011).

RESULTS AND DISCUSSION

The TC concentrations were not significantly different between the BI and NF treatments and between the straw levels. Considering the two treatments, the mean value was 19.52 g kg^{-1}. The TN levels were only 6.4% higher in treatment BI with no difference between the straw levels. The K_2SO_4-C levels were 83% higher in BI than in NF, with no significant differences between the straw levels. The mean values of K_2SO_4-C in BI and NF were, respectively, 67.64 and 37.00 mg kg^{-1}, corresponding to 0.35% and 0.19% of the TC (Table 2).

Table 2. Total C, total N and labile carbon contents (mean ± SE) in the inoculated and with nitrogen fertilizer treatments

Treatments	TC (g kg^{-1})	TN (g kg^{-1})	K_2SO_4-C (mg kg^{-1})
BI	19.42 ± 1.24 a	1.73 ± 0.11 a	67.64 ± 8.00 a
NF	19.62 ± 1.30 a	1.62 ± 0.11 b	37.60 ± 6.60 b

TC, total carbon; TN, total nitrogen; K_2SO_4-C, soil labile carbon extracted with K_2SO_4; BI, sugarcane inoculated with DPGPB; NF, sugarcane with nitrogen fertilizer. Values within the same columm followed by different letters indicate significant differences at $P \leq 0.05$ ($n = 4$).

The absence of a response for TC and the tiny difference in the values for TN between the treatments BI and NF could be associated with the short time during which the treatments were established. Similar results were obtained by other authors, who showed that the total organic C was insensitive to recent management practices (Shafi et al., 2007; Xu et al., 2011). Similarly, Robertson (2003) found higher soil C concentrations in green cane areas in Australia after four to six years, but not in areas recently converted to this management.

The greater content of K_2SO_4-C in treatment BI in relation to NF, can be associated with a possibly greater growth of the roots due to the inoculation, and, as a consequence, a greater concentration of exudate liberated by the rhizosphere. Greater growth of the root system due to the application of plant growth-promoting microrganisms has already been previously demonstrated (Andrade et al., 2024). According to these authors the inoculation of Basilisk grass with PGPB resulted in a 21% increase in root volume. This possibly greater increase in root growth for BI could be associated with the ability of microorganisms of the inoculant to synthesize plant growth regulators such as indolic compounds. These compounds are defined as a group of organic substances with an important role in cell division, elongation, differentiation and root development (Woodward & Bartel, 2005), when its concentration is within the optimal range (Lobo et al., 2023). According to Pereira et al. (2019) all bacteria used in this study for sugarcane inoculation are capable of producing plant growth regulators. Root exudates are low molecular weight organic compounds and represent a significant source of easily degradable organic C (Kuzyakov & Domanski, 2000). The amount of C released from live roots is substantial and estimated to account for up to 20% of the photo-assimilate (Hütsch et al., 2002). Further studies should directly measure root exudate production to confirm the hypothesis of greater release by sugarcane roots in the BI treatment.

Table 3. Basal respiration, fluorescein diacetate hydrolysis and microbial biomass N (mean ±S E) in the inoculated (BI) and nitrogen fertilizer (NF) treatments

Treatments	BR (μg CO_2 day^{-1} g^{-1})	FDA (μg fluorescein h^{-1} g^{-1})
BI	3.46 ± 0.38 a	5.59 ± 0.41 a
NF	2.74 ± 0.52 b	4.50± 0.68 b

BR, basal respiration; FDA, fluorescein diacetate hydrolyses; N_{mic}, microbail biomass N; BI, sugarcane inoculated with DPGPB; NF, sugarcane with nitrogen fertilizer. Values within the same columm followed by different letters indicate significant differences at $P \leq 0.05$ ($n = 4$).

The BR was 21% higher in BI, with a mean value of 3.46 as compared to 2.74 μg^{-1} day^{-1} g^{-1} of soil in the NF treatment. There were no significant differences between the straw levels (Table 3). Similarly, the FDA was, on average, 19% higher in treatment BI with a value of 5.59 as compared to 4.50 μg fluorescein h^{-1} g^{-1} of soil in the NF treatment (Table 3). Although significant differences were obtained in the straw levels for this last parameter, the results were totally inconclusive, so they were not presented. The higher concentration of K_2SO_4-C in BI may have increased the activity of the microorganisms, as verified by the positive correlations between BR and K_2SO_4-C and between FDA hydrolysis and K_2SO_4-C (Table 4). A positive correlation between labile C and FDA hydrolysis was also obtained by Neogi et al. (2014). Whitaker et al. (2014) showed that soil respiration increased to a greater degree with inputs of microbially accessible C compounds than with complex, recalcitrant ones. Greater FDA hydrolysis by DPGPB was also obtained by Rana et al. (2012).

Table 4. Correlations between the variables

	TC	K_2SO_4-C	TN	BR	APA	UA	FDA	GA	N_{mic}
TC	1								
K_2SO_4-C	-	1							
TN	-	-	1						
BR	-	0.54^{**}	-	1					
APA	-	-0.60^{***}	-	-0.48^{**}	1				
UA	-	0.63^{***}	0.47^{**}	-	-	1			
FDA	-	0.68^{***}	-	0.56^{***}	-0.51^{**}	0.44^{*}	1		
GA	-	0.78^{***}	0.55^{**}	0.70^{***}	-0.53^{**}	0.62^{***}	0.63^{***}	1	
N_{mic}	-	0.51^{**}	-	0.42^{*}	-0.41^{*}	0.39^{*}	0.49^{**}	0.51^{**}	1

TC, total C; K_2SO_4-C, labile carbon; TN, total N; BR, basal respiration; APA, acid phosphatase activity; UA, urease activity; FDA, fluorescein diacetate hydrolysis; GA, glucosidase activity; N_{mic}, microbial biomass N. Asterisk represent the significance values, where *, ** and *** indicate $P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ ($n = 32$).

The N_{mic} was 20% higher in BI. The mean values obtained for BI and NF were 16.40 and 13.21 mg kg^{-1} of soil, respectively. The straw levels had no effect on this parameter (Fig. 3). N_{mic} is a microbial process for converting inorganic-N into organic forms and is defined as microbial N immobilization. Soil microrganisms require N to synthesize proteins, nucleic acids, and other cellular components (Geisseler et al., 2010). N immobilization may reduce the risk of losing reactive N to the environment, increasing the synchrony between the supply of N by the soil and that absorbed by the plants. It has been estimated that the mean turnover time of N_{mic} is about 1 to 2 months (Bengtsson et

al., 2003), when it becomes available to the plant by way of mineralization (Cheng et al., 2014). The greater quantity of K_2SO_4-C and, consequently, the greater activity of the microorganisms in the BI treatment, may have been responsible for the accumulation of N into a readily mineralizable pool of organic soil N, that is, greater N immobilization. The positive correlations between N_{mic} and K_2SO_4-C, between N_{mic} and BR and between N_{mic} and FDA, support this assumption (Table 4). These data agree with those obtained by other authors, who reported that the chemical quality of organic matter controlled microbial N immobilization (Cheng et al., 2017; Cao et al., 2021).

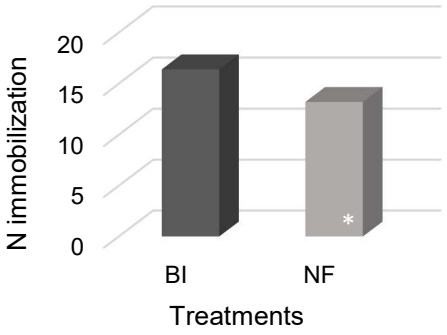

Figure 3. N immobilization (mg kg^{-1}) in the inoculated and with nitrogen fertilizer treatments.
BI, sugarcane inoculated with DPGPB; NF, sugarcane with nitrogen fertilizer. Asterisk represent significant difference between BI and NF treatments at $P \leq 0.05$ ($n = 4$).

The values for APA and UA showed interactions between the BI and NF treatments and the trash levels. Higher APA values were obtained for NF at all trash levels (Fig. 4, I). The APA value correlated negatively with practically all the other parameters evaluated (Table 4). On the other hand, the UA values presented tendencies to be higher for the BI treatment at all trash levels, although significant differences were not found between BI and NF at the levels L0 and L100 (Fig. 4, II).

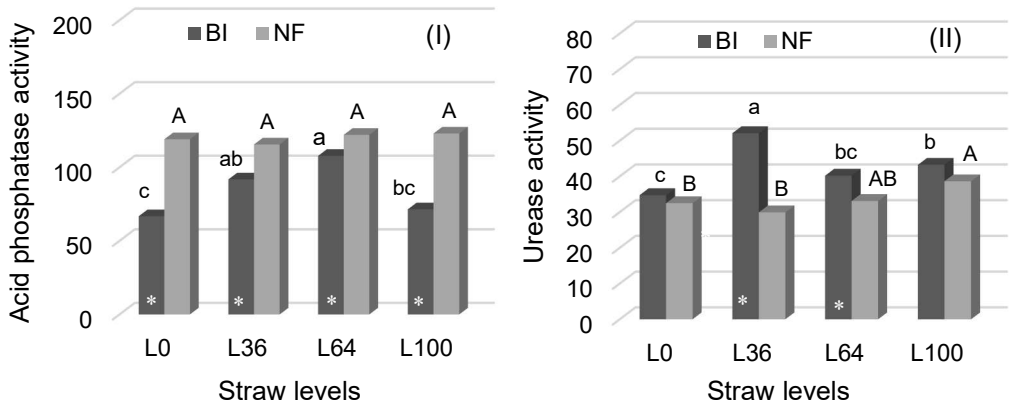

Figure 4. Acid phosphatase (µg *p*-nitrophenol h^{-1} g^{-1} soil) (2-I) and urease (µg NH_3 h^{-1} g^{-1} soil) (2-II) activities in the inoculated and with nitrogen fertilizer application treatments, at the different straw levels.
BI, sugarcane inoculated with DPGPB; NF, sugarcane with nitrogen fertilizer. L0, no straw; L36, 36% straw; L64, 64% straw; L100, 100% straw left on the soil. Values followed by the same small or capital letters for the BI or NF treatments, respectively, did not differ significantly at $P \leq 0.05$ (n =4). Asterisk represent significant difference between BI and NF, within each trash level.

Positive correlations were found between UA and K_2SO_4-C, between UA and FDA hydrolysis and between UA and the TN content (Table 4). The GA values were significantly different between BI and NF, but did not vary between the trash levels (Fig. 5). On average, the GA level was 35% higher for BI, with a mean value of 45.96 µg *p*-nitrophenol h^{-1} g^{-1} of soil as against 29.91 µg *p*-nitrophenol h^{-1} g^{-1} of soil for NF. Positive correlations were found between GA and K_2SO_4-C, GA and TN, GA and BR, GA and FDA and also between GA and UA (Table 4).

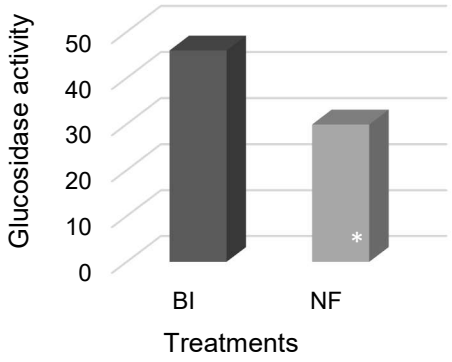

Figure 5. Glucosidase activities (µg *p*-nitrophenol h^{-1} g^{-1} of soil) in the inoculated and with nitrogen fertilizer treatments.

BI, sugarcane inoculated with DPGPB; NF, sugarcane with nitrogen fertilizer. Asterisk represent significant difference between BI and NF treatments at $P \leq 0.05$ ($n = 4$).

Microbes regulate extracellular enzyme production to acquire limiting nutrients, so changes in enzymatic activities may reflect patterns of microbial nutrient limitations and hence nutrient availability (Allison et al., 2011; Burns et al., 2013). The lower APA in BI could be due to the greater availability of P in this treatment, since the production of phosphatase is repressed when the P available in the soil is not a limiting factor (Nannipieri et al., 2011; Wang et al., 2011). According to Farssi et al. (2022) the tolerance of Moroccan alfalfa populations to low P-availability was associated, among other factors, with increased acid phosphatase activity. In this work this could also be demonstrated by the negative correlations between the APA and all the other factors evaluated (Table 4). Since the dose of P applied in the fertilization was the same for all treatments, the interaction of this element with the soil should be considered. Despite the abundance of phosphorus in the soil, in both organic and inorganic forms, it is mostly unavailable for plant uptake due to precipitation with cations in the soil, immobilization, adsorption, and interconversion to the organic form (Kishore et al., 2015; Rawat et al., 2021). As described previously, the possible production of phytohormones by the bacteria in the inoculant could have helped in the expansion of the root system of the sugarcane, allowing them to achieve a greater volume of soil and reach the more distant P, since P is a poorly mobile nutrient (Granada et al., 2018). However, even when the plant roots can physically reach the immobile P in the soil, it is often in a non-soluble form that cannot be taken up by the roots. The root then switches to complementary strategies to improve solubilization, such as the release of selected root exudates to improve P mobilization (Gerke, 2015). Some of the major chemical groups of P-mobilizing root exudates include organic acids, such as amino-acids and fatty acids, with a range of reported biological functions in the plant rhizosphere (Menezes-Blackburn et al., 2018). In the presence of organic acids, the P dissolution rates can be greatly accelerated in the soil, leading to a 10-100-fold higher P concentration in the soil solution, depending on the soil type and organic acid concentration (Gerke, 2015). Another factor to be considered is the possible capacity of the microorganisms of the inoculant to solubilize insoluble phosphorus to the soluble

form, thus increasing the availability of this nutrient in the soil (Hussain et al., 2019), and making it available for use by plants. These factors together, could generate larger amounts of P available in the soil, with a consequent decrease in APA. The increase in APA in BI from level L0 to level L64 could indicate a greater mineralization of organic P with an increase in the straw levels, although still below the values obtained in treatment NF. On the other hand, the decrease in APA at level L100 could be related to a greater liberation of organic acids due to straw mineralization, which could block P sorption sites in the soil and consequently release P to the soil solution (Li et al., 2015) in a concentration that could inhibit acid phosphatase activity, if one considers the effect of nutrient availability on the target enzyme. Thus, the greater values of APA in NF could indicate a smaller availability of P as compared to BI, since in the NF treatments the possible beneficial effects of the DPGPB did not occur, whereas the absence of response to the trash levels in this last treatment could be associated with the short experimental period.

The tendency of increase UA in BI suggests a potential to increase the gross N mineralization rates due to application of the inoculant. Increases in urease activity due to the application of plant-growth promoting microorganisms have already been reported by other authors (Gao et al., 2015; Li et al., 2020; Liu et al., 2020). A smaller urease activity in the treatment with nitrogen fertilizer was also obtained by other authors, who hypothesized that the addition of the end product of the enzymatic reaction (NH_4^+) could suppress urease synthesis (Chang et al., 2007). The positive correlations between UA and K_2SO_4-C and between UA and FDA hydrolysis could indicate that the greater activity of this enzyme in the BI treatment resulted of the greater microorganisms activities due in a greater availability of labile C. An abundant supply of diverse C sources may have resulted in N limitation for the microbial community and hence, a greater production of enzyme to mineralize the organic N. The UA also presented a positive correlation with the total N content, corroborating with the results obtained by Tian et al. (2013). However, it was not clear how the straw levels maintained on the soil were affecting the urease activity.

The beta-glucosidase activity is an indicator used to evaluate the decomposition of organic matter, since its activity depends on the concentration of organic matter in the soil (Moscatelli et al., 2012; Tischer et al., 2015). The greater GA found for the treatment BI could be associated with a greater availability and mineralization of organic matter promoted by the addition of the inoculant, since the total C contents were the same in the two treatments (BI and NF). This can be demonstrated by the greater amounts of labile carbon in the BI treatments. The positive correlations between GA and BR, between GA and the FDA activity and between GA and K_2SO_4-C also confirm this argument. A positive correlation between GA and the labile part of the organic matter was also obtained by Qi et al. (2016). This enzyme may also have contributed to increases in the activities of other enzymes, such as urease. This could occur due the release of low molecular weight sugars, which are energy sources for soil microorganisms (Pathan et al., 2017), causing a greater demand for the N of the soil. The positive correlation between GA and total N could reflect the high microbial potential for the biogeochemical transformation of the N cycle (Brzezińska et al., 2018). A greater GA as a result of inoculating with DPGPB was already described previously (Ramesh et al., 2014).

The highest sugarcane yields were obtained at the levels of L64 and L100 (Table 5). There was no significant difference between BI and NF treatments. Data of cane-plant yields will not be presented, since sugarcane growth was severely hampered due to an atypical dry period. In this year, the yields were on average 62.5 t ha^{-1} for both treatments.

Table 5. Sugarcane yield at the first ratoon (mean ± SE)

Treatments[1)]	Yield (t ha^{-1})
L0	90.79 ± 2.89b
L36	98.18 ± 3.21ab
L64	108.66 ± 4.19a
L100	107.16 ± 3.26a

L0, no straw L36, 36% straw; L64, 64% straw; L100, 100% straw left on the soil. Values followed by the same small letters did not differ significantly at $P \leq 0.05$ ($n = 4$).

The absence of a response in relation to sugarcane productivity between the treatments NF and BI demonstrates the importance of the diazotrophic plant growth-promoting bacteria, principally with respect to the economy in nitrogen fertilizers. Atypical environmental conditions occurred during the cane-plant cultivation that resulted in low yields (on average 62.5 t ha^{-1}) for both treatments. Since nitrogen fertilizer was not applied as side dressing in BI treatment, this demonstrates that even under adverse conditions the inoculation can result in nitrogen fertilizer savings. During the cane-plant cultivation the inoculation with DPGPB replaced 100 kg N ha^{-1}. At the first ratoon the sugarcane yield was similar to that induced by 120 kg N ha^{-1}. According to Schultz et al. (2014), the increase in yield of the sugarcane variety RB72454 in the cane-plant and first ratoon crops after inoculation with diazotrophic bacteria, was comparable to that induced by fertilization with 120 kg N ha^{-1}. The results also show the adequacy of removing part of the straw to produce second generation ethanol, since there were no differences in terms of productivity between treatments L64 and L100. According to Aquino et al. (2015), for a sugarcane cycle occurring during a drought, a quantity of 10 Mg ha^{-1} of trash (50%) was sufficient to promote improvements in the root system and in the productivity of the culture, and above this value, there was no significant response. Thus, the rest of the trash could be employed in other sectors without prejudice to the sustainability of the culture system. In the same way, Melo et al. (2020) suggested that moderate trash removal was sufficient to sustain the physical conditions of the soil for root growth and improve the sugarcane yield, part of the trash being removed for industrial purposes.

The results obtained in this work did not allow for a clear inference about the effects of maintaining different amounts of sugarcane trash on the soil, with respect to the soil enzymatic enzymatic and microbiological activities. Maybe the effects of the quantities of trash were, in general, inconclusive, or non-significant for the majority of parameters evaluated, because of the short experimental period. This occurred both for the plots that received nitrogen fertilizer and for those inoculated with DPGPB. Nevertheless, some of the results allow one to discuss the importance of determined treatments to obtain a more sustainable production of sugarcane. The tendencies for smaller APA values for BI leads us to presume that the DPGPB could have an important role in the phosphate nutrition of the sugarcane. Rosa et al. (2022) demonstrated that the inoculation of sugarcane with *Azospirillum brasilense* and *Bacillus subtilis* associated with 45 kg ha^{-1} of P_2O_5 resulted in a savings of 75% of the recommended P_2O_5 rate. In the same way, Fernandes et al. (2023) demonstrated that the inoculation of sugarcane with PGPB was beneficial for the

sugarcane crop, reducing phosphate fertilization and increasing productivity. However, these results were obtained from the inoculation of sugarcane with other bacterial species and not with the microorganisms used in the presente study, which are recommended for inoculation of sugarcane in Brazil. Futhermore, in those studies the acid phosphatase activity was not determined. To confirm our results, additional experiments using the inoculant used in this study should be tested with different doses of phosphorus, including the determination of APA. For greater clarity of the role of DPGPB in the phosphate nutrition of sugarcane, further experiments should also include an analysis of P in the plant associated with an analysis of P available in the soil. Typically, soils used for sugarcane cultivation have a high phosphorus (P) fixation capacity, and therefore the application of large amounts of soluble P fertilizer are required to help supply the P levels that allow for an adequate crop productivity (Roy et al., 2016). Sugarcane receives 20% of the total consumption of inorganic phosphate fertilizers used in Brazil (FAOSTAT, 2017). The P input via mineral fertilizer for sugarcane was 1263 kg ha^{-1}, with a corresponding crop P offtake of 420 kg ha^{-1} in Brazil for the 1967–2016 period (Pavinato et al., 2020). A sudden increase of 800% in the price for inorganic phosphate fertilizers in 2008 caused serious concern about the depletion of this element (Mew, 2016). If one considers the environmental problems resulting from the application of phosphate fertilizers and that the reserves of P are finite and non-renewable (Mutale-joan et al., 2024), the use of these fertilizers is not in agreement with the principles of sustainable agriculture. The decrease or lack of nitrogen fertilization, as observed in this work, associated with a possible decrease in phosphate fertilization in areas where the plants were inoculated with DPGPB, apart from decreasing the sugarcane production costs, they contribute to a decrease in the environmental problems resulting from the use of these two fertilizers. Nitrogen fertilizers can be lost to the atmosphere through ammonia volatilization and oxid nitrous emission and also by nitrate leaching causing different harmful effects on the environment. On the other hand, inappropriate or excessive use of P fertilizers has been linked to the heavy metal contamination in soils, and P runoff that can contribute to the eutrophication of water bodies (Chien et al., 2009). Logically, long-term studies should be carried out, since over the years there will be an effect resulting from the decomposition of the straw in liberating nutrients to the soil, by way of microbial activity. Logically, this study should continue using diferente sugarcane genotypes, since they present different responses to diazotrophic bacteria association (Urquiaga, 2012; Martins et al., 2020). It is also importante to emphasize that sugarcane cultivation areas in Brazil cover a wide variety of edaphoclimatic conditions and therefore experiments should be carried out in different sugarcane production locations. It would also be interesting to study how DPGPB can modify the soil microbial community and how this would affect the long-term efficiency of the inoculant or the microbial properties of the soil. Another important point to be made in this study refers to the soil enrichment with labile C in the treatment that received the inoculation. Although there is intense research being carried out concerning the effect of DPGPB on the growth and productivity of sugarcane, no studies were found relating these types of microorganisms with the labile C fractions in the soil. Due to the importance of the labile carbon fraction for the microrganismos acitivies and for the formation of more stable organic soil C, it would be interesting that other labile C

assessment techniques were used in order to obtain a better understanding of the effects of DPGPB on sugarcane growth and productivity. Different fractions have been suggested as being the most sensitive to management and various methodologies have been applied, hampering comparisons with labile carbon (Poeplau et al., 2018).

CONCLUSION

This study showed that the DPGPB can have a relevant role in the soil microbiological and enzymatic activities in area cultivated with sugarcane. The absence of effects caused by the levels of straw on many of these factors, both for BI and for NF, could have been associated with the short period of experimentation. The enzymatic activies (APA, UR and GA), FDA hydrolysis, basal respiration and microbial biomass N were good indicators of the beneficial effects of inoculation of sugarcane with DPGPB. Along with these parameters the labile carbon content was essential for evaluating the effects of the inoculant on the soil microorganisms and enzymatic activities. The absence of a differential response for sugarcane yield between BI and NF shows that the application of bacteria as plant growth promoters is a sustainable alternative to mitigate the use of nitrogen chemical fertilization and possibly phosphate fertilizer, reducing negative economic and environmental impacts of these two types of fertilizer.

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